MECHANISMS AFFECTING SPREAD IN TUBERCULOSIS*

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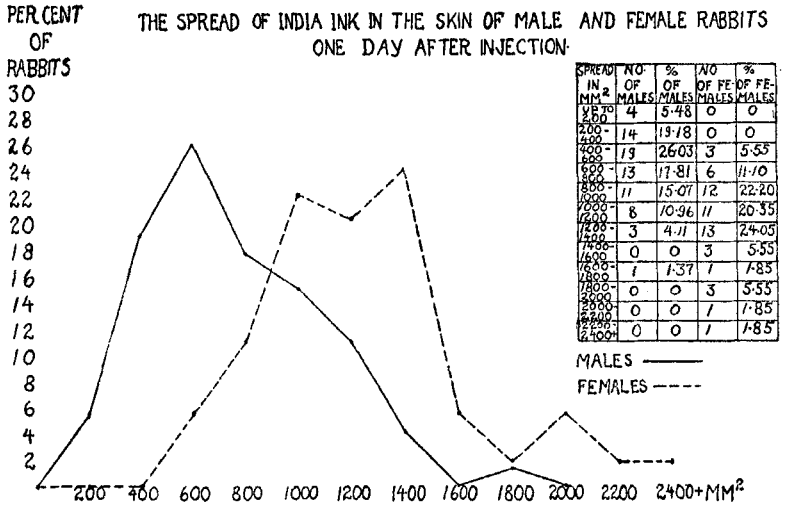
In a study[1] on the native resistance of inbred rabbit families of varying hereditary resistance to tuberculosis, it was found that the fundamental variant in the pathogenesis of the disease developed by these families was the degree of localization of the infection at the portal of entry. Families of high resistance effectively limited the process to the lungs, if the tuberculosis was of natural respiratory origin, with little or no dissemination of the disease by lymphogenous or hematogenous routes (PLATE 1, a). In families of low resistance, on the other hand, the primary tuberculosis in the lung progressed rapidly and was soon widely disseminated through the



SPREAD IN MM²	NO. OF MALES	% OF MALES	NO OF FEMALES	% OF FEMALES
UP TO 200	4	5·48	0	0
200–400	14	19·18	0	0
400–600	19	26·03	3	5·55
600–800	13	17·81	6	11·10
800–1000	11	15·07	12	22·20
1000–1200	8	10·96	11	20·35
1200–1400	3	4·11	13	24·05
1400–1600	0	0	3	5·55
1600–1800	1	1·37	1	1·85
1800–2000	0	0	3	5·55
2000–2200	0	0	1	1·85
2200–2400+	0	0	1	1·85

FIGURE 1. The spread of India ink in the skin of male and female rabbits one day after injection.

body by the vascular system (PLATE 1, b). Since bacterial infection takes place chiefly in the connective tissue, it was thought that the permeability of this tissue to particulate matter might be one of the factors in this resistance. In fact, the spread of India ink in the skin of the most resistant family (PLATE 1, c-1) was greatly restricted as compared with that of the most susceptible family (PLATE 1, c-2), though there was no strict correlation between resistance and tissue permeability. That the spread of particles in the connective tissue might be under the influence of naturally occurring sex hormones was suggested by the observation[2] that male rabbits limited this spread more effectively than females (FIGURE 1). Sprunt[3] has shown

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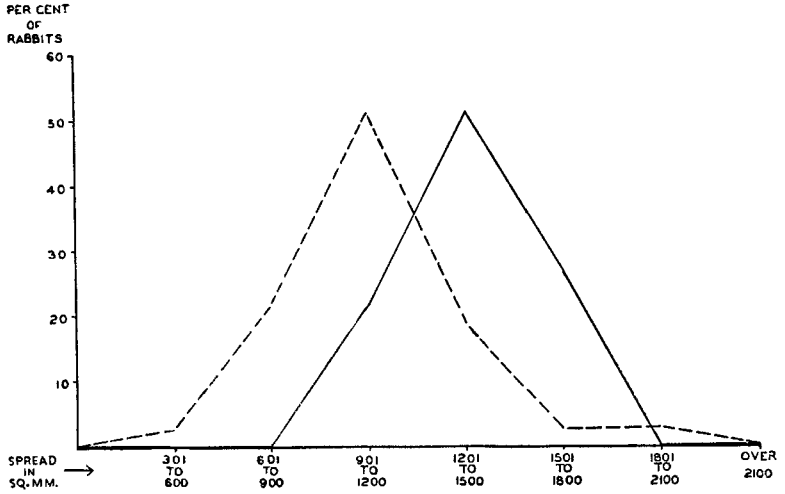

FIGURE 2. Effect of chorionic gonadotropin on the spread of India ink in the skin of female rabbits twenty-four hours after the injection of the dye. The broken line represents the curve of spread before and the solid line that after the administration of gonadotropin.

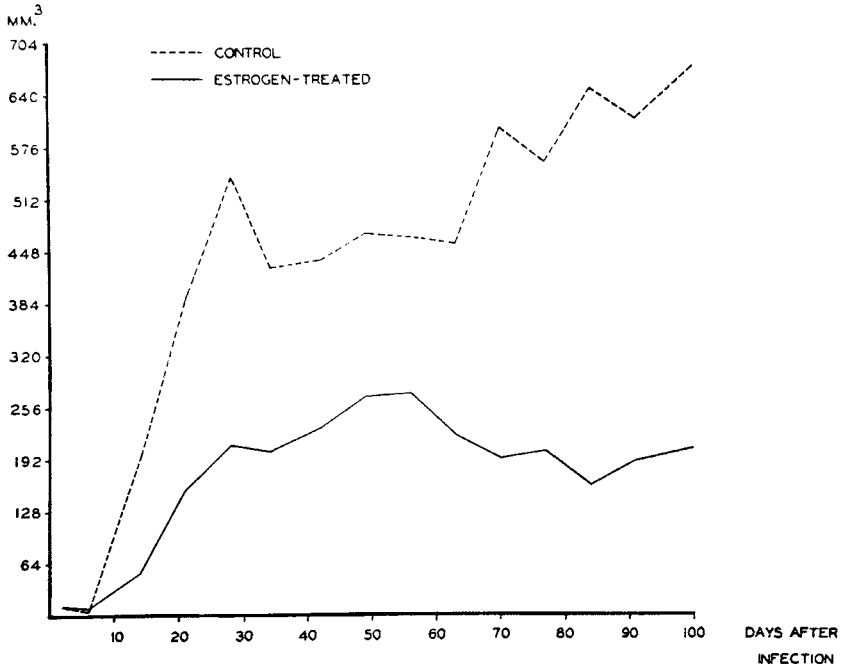

FIGURE 3. Average volume of local lesion in estrogen-treated and control litter mates of the C family (Experiment of 1944–45).

that estrogen restricted the spread of India ink in the skin. On the other hand, it was found in our laboratory that the intravenous injection of the luteinizing hormone, chorionic gonadotropin,[4] enhanced this spread (FIGURE 2).

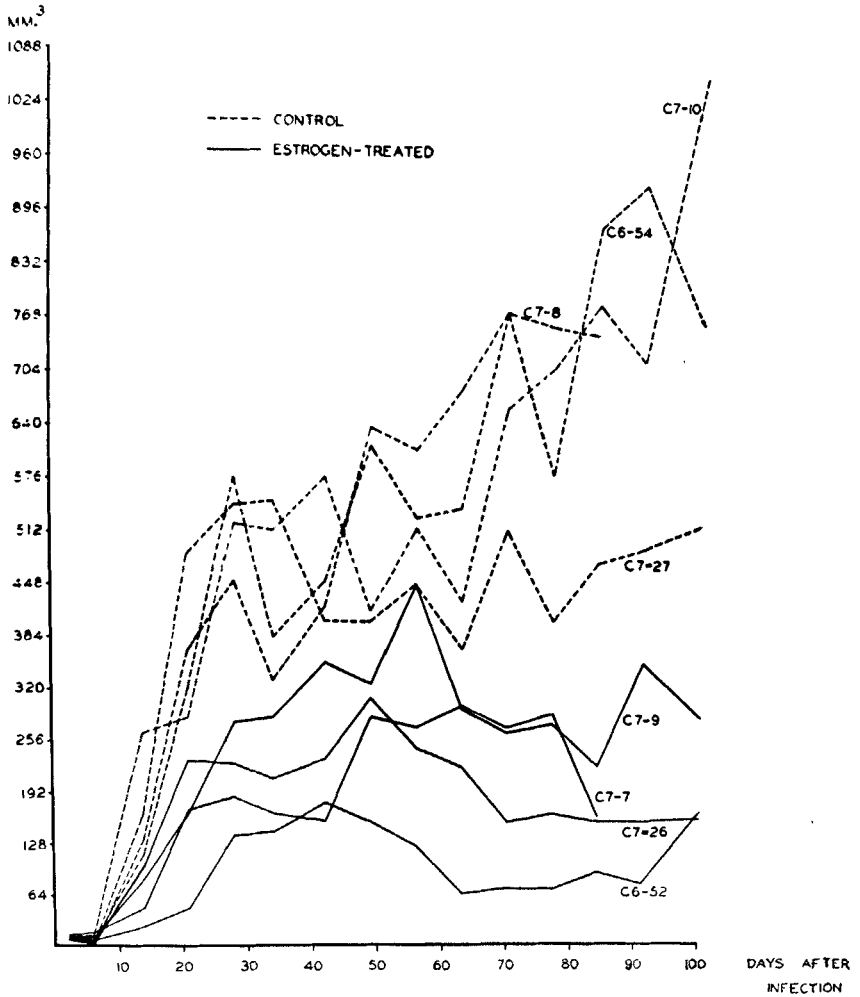

FIGURE 4. Individual volumes of local lesions in estrogen-treated and control litter mates of the C family (Experiment of 1944–1945).

In a study of the effect of these sex hormones on the tuberculous process, it was found that, in highly inbred, sexually mature rabbits of similar generic resistance to tuberculosis estrogen, in large doses, they uniformly retarded the progress of the disease at the site of intracutaneous inoculation in the skin (FIGURES 3 and 4 and PLATE 1, d & e), diminished the extent of the disease in the internal organs (PLATE 1, f & g), and suppressed to a

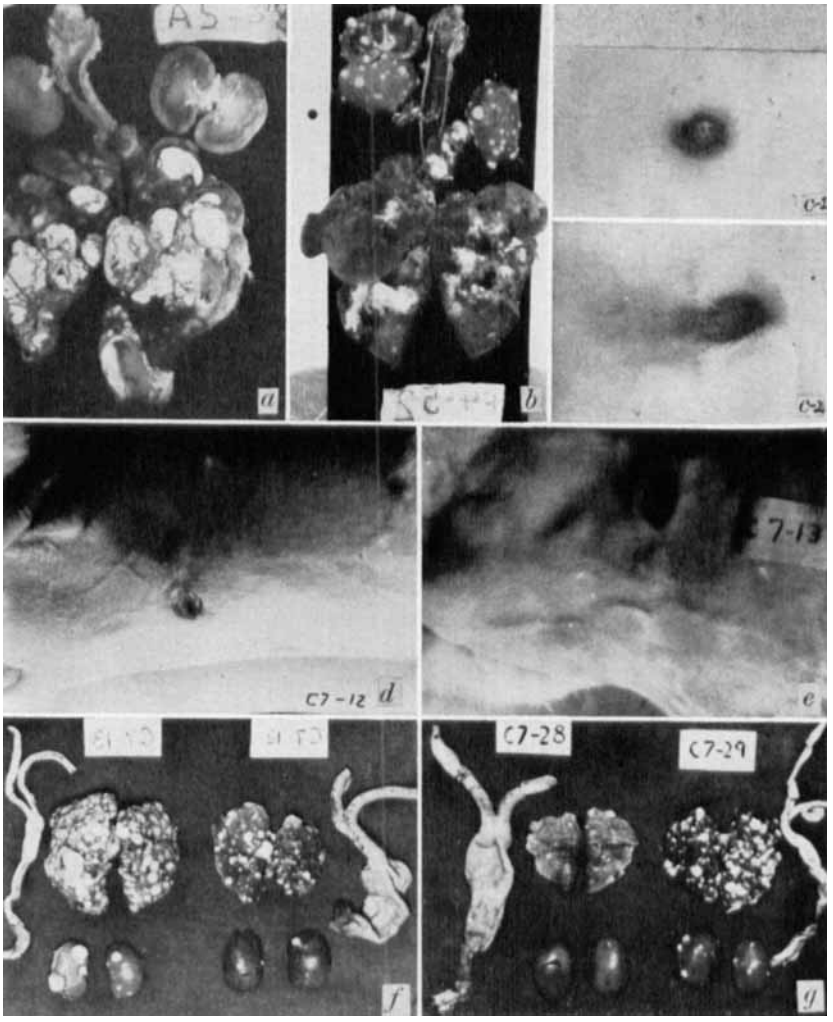

PLATE 1. *a*. Organs of resistant rabbit A 5-38. Rabbit died 161 days after a single inhalation of 27 droplet nuclei of bovine bacilli. Strictly localized ulcerative pulmonary phthisis. There is no lymphogenous or hematogenous dissemination of the disease beyond the portal of entry, the lung.

b. Organs of susceptible rabbit F4-52. This rabbit was exposed simultaneously with A5-38 shown in "*a*" and inhaled the same number of tubercle bacilli. This "F" rabbit died 102 days after exposure from multiple foci of caseous pneumonia and extensive dissemination of the disease from the portal of entry by lymphogenous and hematogenous routes.

c-1. The spread of 0.5 cc. India ink in the skin of resistant rabbit A 4-16 on the fifteenth day after intracutaneous injection.

c-2. The spread of India ink in the skin of susceptible rabbit F3-28 on the fifteenth day after the intracutaneous injection of the same amount of India ink as in rabbit A 4-16, shown in "*c*-1."

d. The lesion at site of intracutaneous inoculation in the estrogen-treated rabbit C7-12 on the fourteenth week of infection. Note hypertrophy of nipple due to estrogen in the upper half of the figure, opposite the ulcer.

e. The large spreading lesion at site of intracutaneous inoculation on the fourteenth week after infection in the C7-13, an untreated litter-mate of C7-12 shown in "*d*." The small nipple is seen below the label.

f. The lungs, kidneys, and uterus of the estrogen-treated rabbit C7-12 and the same organs of its untreated litter-mate, C7-13.

g. The lungs, kidneys, and uterus of the estrogen-treated rabbit C7-28 and the same organs of its untreated litter-mate, C7-29.

considerable degree its dissemination in the body as compared with that in untreated litter-mates. These results are in agreement with those of Sprunt and McDearman,[5] Foley and Aycock,[6] and Von Haam and Rosenfeld,[7] who found that estrogen exerts a protective influence against vaccine virus, the streptococci, and the pneumococcus, respectively. By contrast, the induction of successive crops of corpora lutea in the ovary during the early phases of the infection, by the intravenous injection of 0.02 to 0.2 mg. chorionic gonadotropin every tenth day, enhanced the disease at the portal of entry in

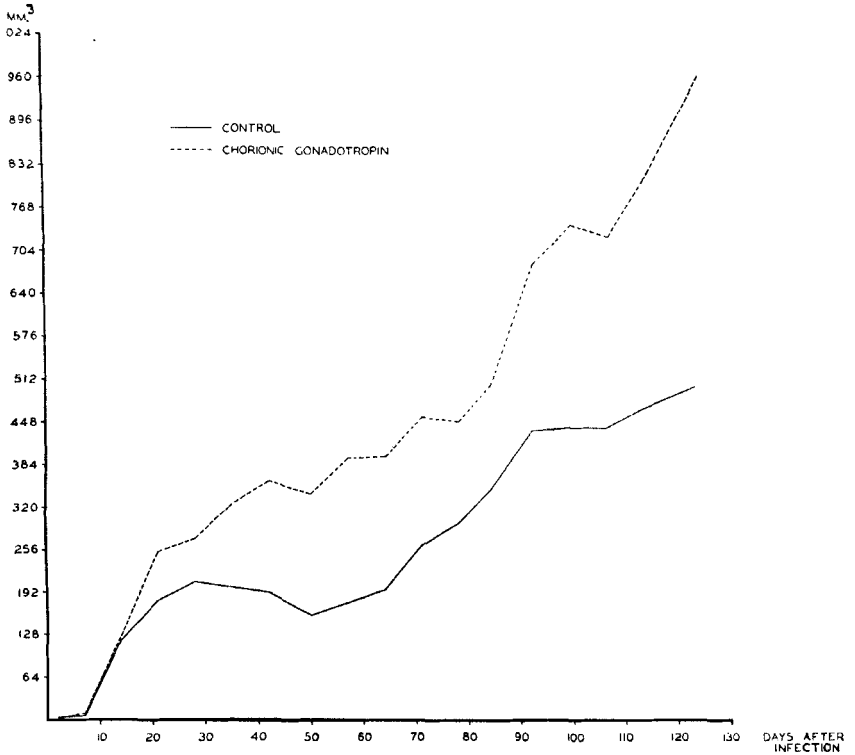

FIGURE 5. Average volume of local lesion in chorionic gonadotropin-treated and control litter mates of the A family.

the skin (FIGURE 5 and PLATE 2, a & b) and increased its spread to the viscera. These observations are in harmony with those of Thomas and Duran-Reynals,[8] who accelerated the progress of tuberculosis with hyaluronidase.

To what extent these data apply to human tuberculosis is problematical. It is well known that the incidence of tuberculous infection as distinguished from tuberculous disease increases regularly with age. However, the mortality and morbidity of the disease rises disproportionately synchronous with the onset of puberty, particularly in the female. This sudden change can be understood on the hypothesis that the sex hormones exercise the same effects in man and rabbits. With the onset of the menstrual cycle, a latent tuber-

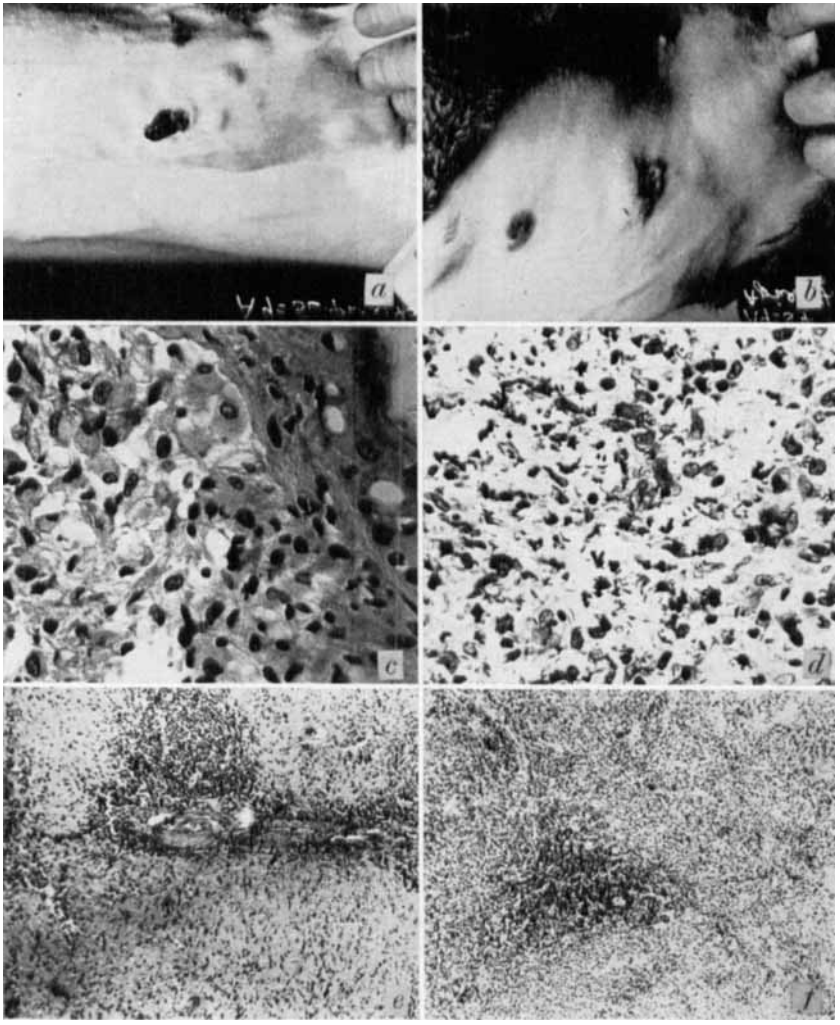

PLATE 2. *a*. The lesion at site of intracutaneous inoculation and its spread in rabbit A9-56 under the influence of periodic injections of chorionic gonadotropin, 128 days after infection.

b. The lesion at site of intracutaneous inoculation and its delimitation in A9-54, an untreated litter-mate of A9-56, shown in "*a*" 128 days after infection.

c. The wall of a small sinus remaining in the skin of resistant rabbit A 5-9 at death, one hundred and eighty-nine days after an intracutaneous inoculation of 0.2 mg. Ravenel strain of bovine-type bacilli. Mature epithelioid cells rarely contain any bacilli which have been largely destroyed. Only one micro-organism can be seen within such an epithelioid cell, at about the center of the figure.

d. Lesion at site of intracutaneous inoculation in susceptible rabbit F4-7 at death, on the one hundred and forty-ninth day following an identical inoculation given to A5-9, the corresponding lesion of which is shown in "c." Intact mononuclears contain numerous tubercle bacilli which these cells failed to destroy.

e. Spleen of control rabbit C7-13. The pulp is largely replaced by amyloid degeneration.

f. Spleen of estrogen-treated litter-mate C7-12. There is no amyloid degeneration.

culous focus is alternately under the influence of estrogen in the first portion of the cycle and under the corpus luteum, for some time preceding menstruation, in the latter half. It is conceivable that estrogen retards the spread of

the disease in the first portion of the cycle, as it does in rabbits, whereas, in the latter half, the reduction of estrogen together with the activity of the corpus luteum induce a spurt of spread in the heretofore dormant focus in the same manner as the corpora lutea induced by gonadotropin increase the dissemination of the disease. However, since the estrogen used in these experiments, 0.5 mg. α estradiol dipropionate subcutaneously once weekly, far exceeds the physiological levels, it is questionable whether these observations can be directly applied to the human disease.

As to the mode of action of these hormones on the tuberculous process, it may be said that estrogen has no retarding effect on the growth of tubercle bacilli in the body, nor does it exercise an enhancing effect on their destruction (TABLE 1), whereas, in the naturally resistant animal, the mononuclear

TABLE 1

FATE OF TUBERCLE BACILLI AT THE PORTAL OF ENTRY AND IN THE DRAINING NODES AND KIDNEYS OF ESTROGEN-TREATED AND NORMAL RABBITS

Rabbit number		*Days after infection*	*Number of viable tubercle bacilli on culture*					
			local lesion		*draining nodes*		*kidney*	
experimental; age in months at time of infection	*control; age in months at time of infection*		*experimental*	*control*	*experimental*	*control*	*experimental*	*control*
A9-98; 17	A9-99; 17	2	80	624	750	1,225	1	1
A9-102; 17	A9-103; 17	7	1,140	1,116	29,800	14,520	0	0
A9-135; 4	A9-138; 4	14	56,000	43,000	260,000	—	0	?
A10-7; 4	A10-10; 4	28	11,000	2,000	106,500	133,500	0	0

phagocytes acquire the power to destroy tubercle bacilli more rapidly and effectively than the cells of the susceptible animal (PLATE 2, c & d).

Furthermore, while estrogen markedly reduces the inflammatory response of the skin to tuberculin in rabbits sensitized by active tuberculosis or by treatment with heat-killed tubercle bacilli (FIGURES 6 and 7), this is not due to a depression of the allergizing mechanism by the hormone. As soon as the estrogen is removed, the sensitivity returns to its full intensity (FIGURE 8). The hormone merely masks an existing undiminished allergic skin sensitivity, for estrogen reduces the inflammatory response of the skin to bacterial toxic agents in general (TABLE 2) and, among these in particular, to tuberculin, which is toxic to sensitized animals. Again, chorionic gonadotropin does not influence the rate and intensity of development of allergic irritability of inbred rabbits treated with heat-killed tubercle bacilli (FIGURE 9). It may be remembered that naturally resistant rabbits tend to develop allergic irritability more rapidly and intensely than susceptible animals (FIGURE 10).[1] Furthermore, neither estrogen nor gonadotropin materially affect antibody production (FIGURES 11 and 12).[9] In this relation, it may be recalled that the genetically most resistant family produced antibodies

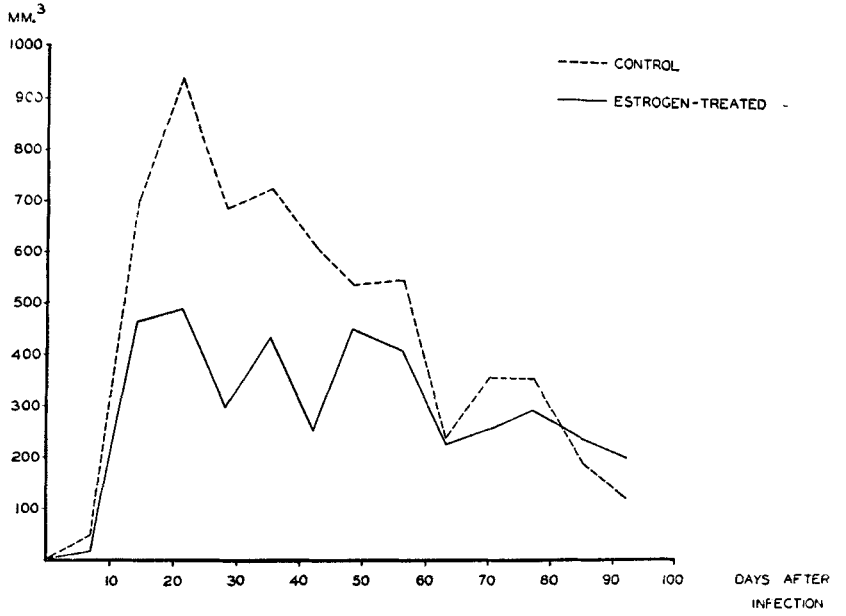

FIGURE 6. Average volume of tuberculin reaction during course of infection in estrogen-treated and control litter mates of the C family (Experiment of 1945-1946).

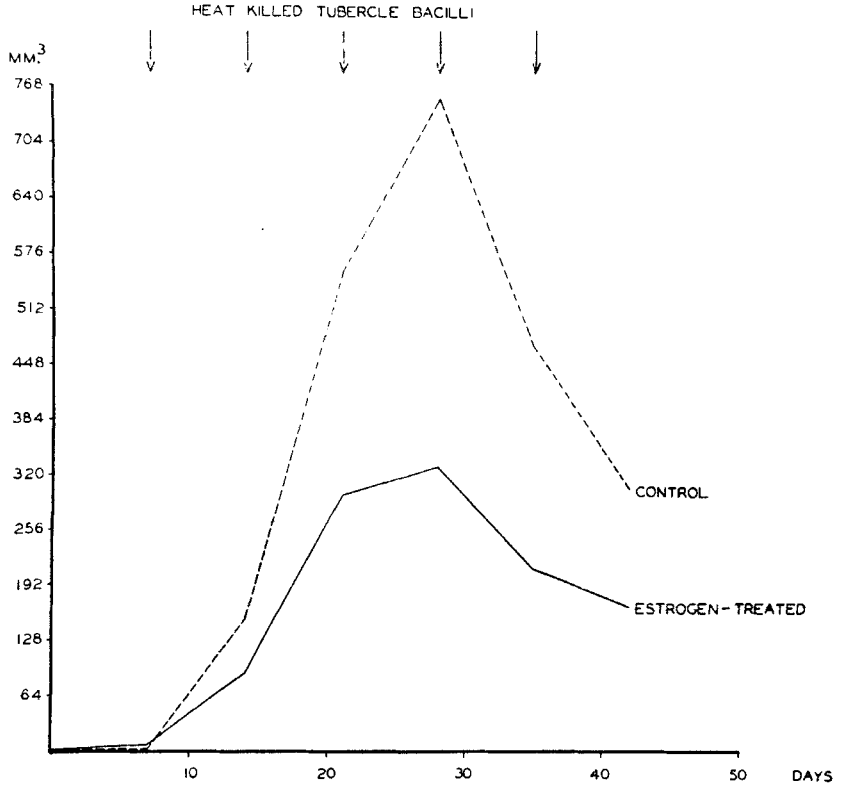

FIGURE 7. Average volume of tuberculin reaction after sensitization with heat-killed tubercle bacilli in estrogen-treated and control inbred rabbits (Experiment of 1946).

much more rapidly and intensely than the most susceptible family on stimulation with heat-killed tubercle bacilli (FIGURE 13).[1]

Thus, the mechanism whereby estrogen tends to localize the infection and gonadotropin tends to spread it differs in important respects from that by which naturally resistant rabbits restrict the infection at the portal of entry and naturally susceptible animals permit its dissemination.

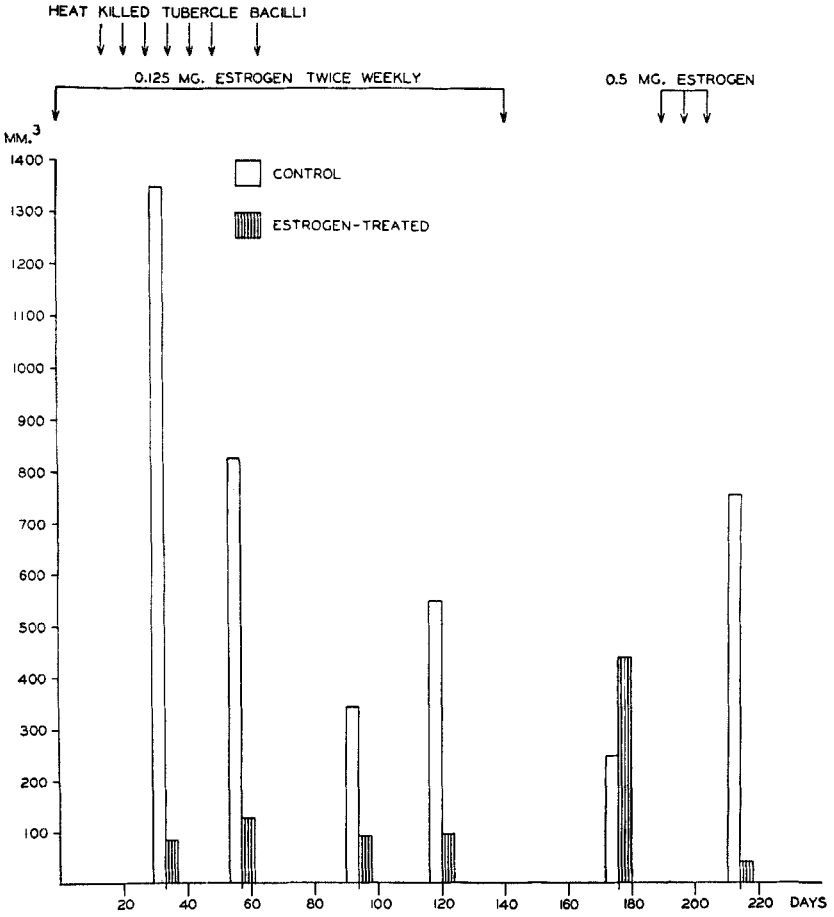

FIGURE 8. The effect of estrogen, its withdrawal and readministration on the tuberculin skin allergy of rabbits sensitized with heat-killed tubercle bacilli.

There is some evidence suggesting that estrogens induce the adaptation syndrome of Selye.[10] In mice, the administration of estrogen in amounts comparable to that used in rabbits induced hypertrophy of the adrenal cortex and atrophy of the thymus (TABLE 3). In rabbits, there is a reduction of circulating lymphocytes (TABLE 4), but no increase in adrenal weights nor any alteration in cortical structure as revealed by lipid stains. Furthermore, no definitive evidence was obtained by the study of liver glycogen

TABLE 2

THE EFFECT OF ESTROGEN ON THE INFLAMMATORY IRRITABILITY OF THE SKIN TO HEAT-KILLED TUBERCLE BACILLI AND TO PERTUSSIS ENDOTOXIN

Response to heat-killed tubercle bacilli			*Response to pertussis endotoxin*		
group	*rabbit number*	*volume inflammation in mm³*	*group*	*rabbit number*	*volume of inflammation in mm³*
Treated with estrogen	V 30	44	Treated with estrogen	V 30	76
	V 366	34		V 366	81
	V 784	54		V 784	144
	V 267	26		V 267	28
	V 414	39		V 414	50
Average		39			76
Untreated controls	T 33	69	Untreated controls	T 33	68
	V 685	140		V 685	392
	V 368	85		V 368	432
	V 57	76		V 57	118
	S 376	97		S 376	54
Average		93			213

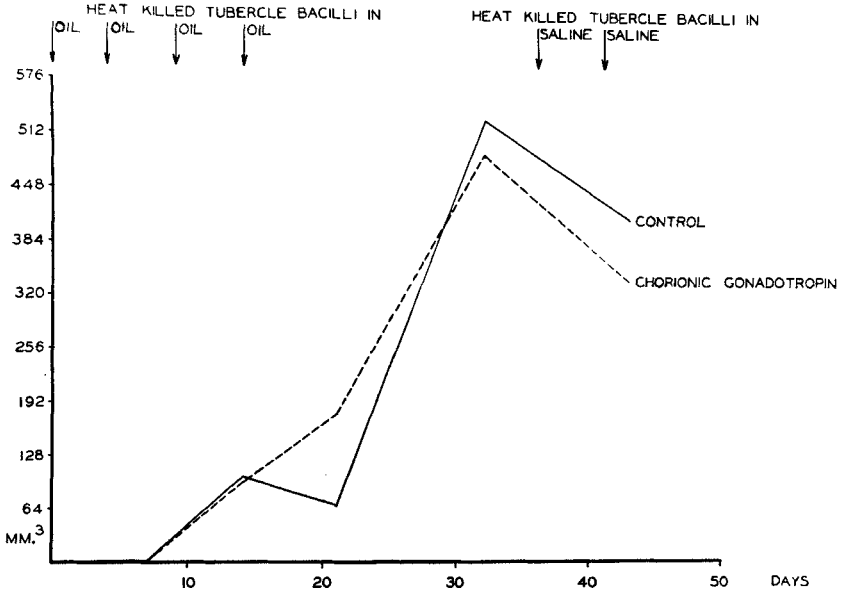

FIGURE 9. Average volume of tuberculin reaction after sensitization with heat-killed tubercle bacilli in chorionic, gonadotropin-treated, and control rabbits of the highly inbred family A.

deposits of increased adrenocortical function as a result of estrogen treatment. The failure of antibody production to be affected by either estrogen or gonadotropin in these rabbits would also suggest that the cortical hormone

implicated in antibody production by Chase, White, and Dougherty[11] is not involved in this process. Thus, no direct evidence was obtained that estrogen retards the tuberculous process *via* its effects on the adrenal cortex. As may be seen in TABLE 3, however, there is clear evidence that tuberculosis *per se* induces a marked hypertrophy of the adrenals of rabbits.

One of the most impressive differences between estrogen-treated and control tuberculous litter mates was the marked reduction in amyloid degeneration in the spleen, which is characteristic of rabbits dying from chronic tuberculosis (PLATE 2, e & f). This could not be accounted for by the less extensive disease in the hormone-treated animals, for frequently the difference in the extent of the disease was only moderate in the two litter mates,

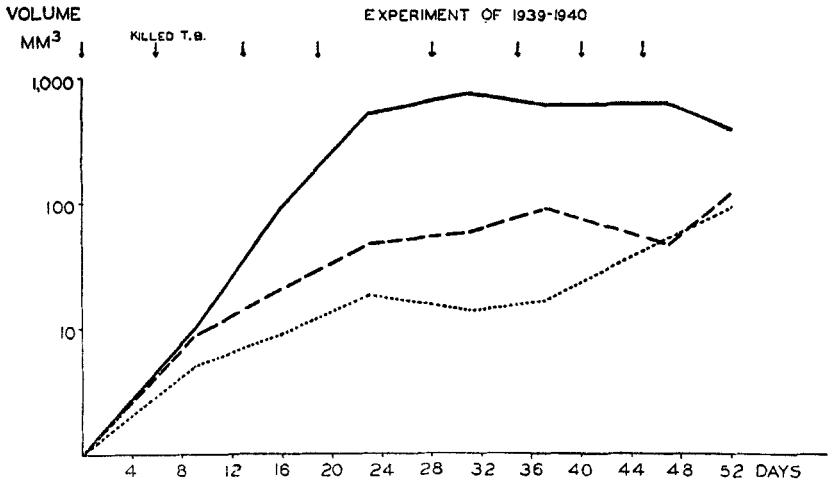

FIGURE 10. The average volume of the tuberculin reaction in three families of varying genetic resistance to tuberculosis during the course of identical injections of heat-killed tubercle bacilli. (—resistant; — — — intermediate; . . . . susceptible.)

whereas the amyloid degeneration was extensive in the control animal and completely absent in the estrogen-treated rabbit. In the same category is the observation that the incidence of degenerative changes in the adrenals, such as hemorrhage, leucocytic infiltration, and focal necrosis is definitely reduced in the estrogen-treated rabbits.

While these effects of estrogen are significant, they do not explain the marked retarding effect of estrogen on the progress of tuberculosis at the portal of entry in the skin nor its enhancement by chorionic gonadotropin, which is apparent in the first few weeks after infection and long before these changes are apparent in the internal organs. The explanation of the opposite effects of these two hormones must be sought in their effects on the permeability of the connective tissue which determines the course of events at the very beginning of the infection.

It was shown by Zuckerman[12] and his collaborators that estrogen increases the water of the skin of mice. Taylor and Sprunt[13] also presented evidence

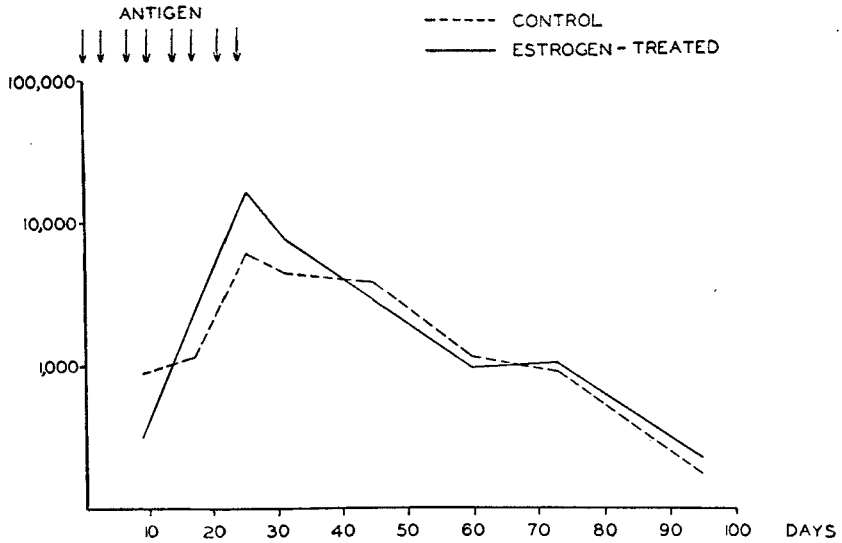

FIGURE 11. The geometric mean of the agglutinin titers or 5 estrogen-treated and 5 control rabbits vaccinated wity thyphod bacilli.

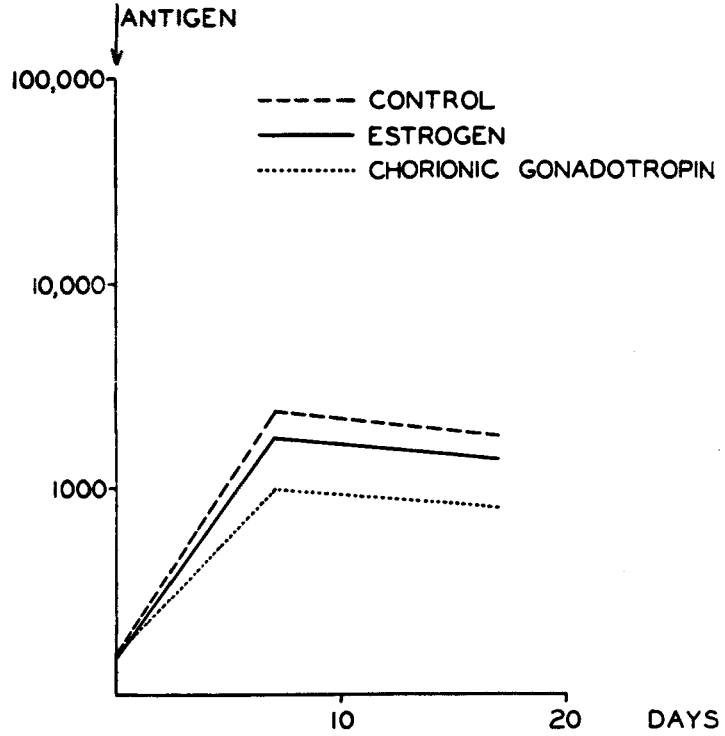

FIGURE 12. The geometric mean of the agglutinin titers of 5 control rabbits, 5 estrogen-treated, and 5 chorionic gonadotropin-treated animals, respectively, all given a single injection of killed dysentery bacilli. (—resistant; – – –intermediate; ....susceptible.)

suggesting that the extracellular fluid in the skin of rabbits is increased by estrogen. Furthermore, Chain and Duthie[14] claim that in the sex skin of monkeys a substance similar to hyaluronic acid accumulates as a result of the hormone. All this would suggest that, as a result of the swelling of the connective tissue elements due to estrogen, their turgescence is increased and their permeability to particles reduced.

That the tautness of the connective tissue may be an important factor in its permeability was suggested by the following observations. Hyaluronidase enhanced the spread of particles to a greater extent in the skin of estro-

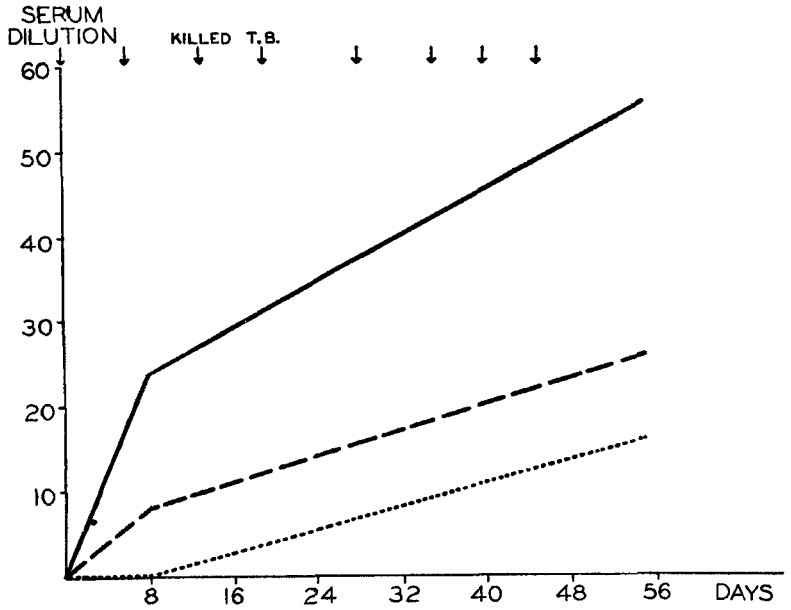

FIGURE 13. The average agglutinin titre in three families of varying genetic resistance to tuberculosi during the course of identical injections of heat-killed tubercle bacilli.

gen-treated rabbits than in that of litter mates under the influence of gonadotropin (TABLE 5). This may be due to a number of variables, but the simplest explanation is afforded by the work of Hechter.[16] He demonstrated that the diffusion of hyaluronidase in the tissue is very low and that, as a consequence, the spreading effect of hyaluronidase is determined not only by its concentration but also by the volume and pressure of the intradermal injection. The greater the intradermal pressure at the point of introduction of the enzyme, the more widely will the enzyme spread in the tissues and the greater will be the extent of the hydrolysis of the hyaluronic-acid matrix of the skin. Since estrogen reduces permeability and gonadotropin enhances it, it is clear that a given volume of fluid injected into the skin of an estrogen-treated rabbit will be under greater pressure than in an animal under the influence of the gonadotropin. Therefore, the hyaluronidase will diffuse to a greater degree in estrogen-treated than in gonadotropin-treated animals. This suggests that estrogen increases and gonadotropin reduces the turgidity

of its elements and thus play a role in altering the permeability of the connective tissue.

TABLE 3

THE EFFECT OF ESTROGEN ON THE WEIGHT OF THE ADRENALS OF NORMAL AND TUBERCULOUS ANIMALS AFTER VARYING INTERVALS OF TREATMENT

Animals used	*Number of weekly injections*	*Number of animals and their average weight in grams*				*Weight of adrenals in mg. per 100 grams of body weight*		
		control		*treated*		*control*	*treated*	*"p" value of difference*
		number	*weight*	*number*	*weight*			
Mice	3	12	18.9	10	19.1	38 ± 2.3*	51 ± 3.8†	0.004
Normal rabbits	3 to 8	9	3390	9	3170	10.8 ± 1.0	12.5 ± 1.4	0.166
Tuberculous rabbits	15 or more	11	2320	11	2630	23.4 ± 2.5	17.1 ± 1.7	0.024

* Width of cortex—2.2 ± 0.05.
† Width of cortex—2.9 ± 0.19. "p" value of difference between the width of the cortex of the adrenals in normal and estrogen-treated mice = 0.002.

TABLE 4

THE EFFECT OF ESTROGEN ON THE CIRCULATING LYMPHOCYTES OF NORMAL AND TUBERCULOUS INBRED RABBIT LITTER MATES

Group	*Rabbit number*	*Absolute number of lymphocytes per mm³**			
		before infection		*after infection*	
		prior to estrogen treatment of group 2	*20 days after estrogen treatment of group 2*	*11 days after infection*	*103 days after infection*
(*1*) Not treated with estrogen	C8-2	4392	4520	4579	4704
	C8-17	4858	4185	3750	5088
	C8-19	4772	3738	5481	7627
	C8-23	5236	3953	2525	4630
Average		4690	4049	4084	5512
(*2*) Treated with estrogen	C8-1	4720	2150	2208	4802
	C8-16	4982	4683	1515	1340
	C8-18	4862	2247	1980	3032
	C8-24	2215	2072	972	2480
Average		4197	2788	1669	2914

* Per cubic millimeter of free-flowing venous blood.

There was much circumstantial evidence to suggest that the permeability of the blood vessels may be oppositely affected by these two hormones. The attempt was made to determine whether estrogen and gonadotropin affect

this property, using the method of Menkin.[15] An exudate containing "leucotaxin" was injected intracutaneously into normal, estrogen-treated and

TABLE 5

THE EFFECT OF HYALURONIDASE ON THE SPREAD OF INDIA INK IN THE SKIN OF NORMAL ESTROGEN-TREATED AND GONADOTROPIN-TREATED LITTER MATES OF THE INBRED RABBIT FAMILY "A"

Group	*Number of rabbits*	*Spread of India Ink in mm²*					*spread of hemoglobin in mm²*		
		without hyaluronidase	*with hyaluronidase 1 γ/cc.*	*5 γ/cc.*	*average of 10 observations*	*increment of spread for both doses*	*without hyaluronidase*	*with 5 γ of hyaluronidase/cc.*	*increment of spread*
Normal	5	221	233	283	259	1.2 ± 0.06	285	595	2.1 ± 0.11
Estrogen ...	5	164	194	209	202	1.3 ± 0.05	224	518	2.3 ± 0.11
Gonadotropin	5	261	275	295	283	1.1 ± 0.03	580	636	2.0 ± 0.12

"p" value of difference between the effect of the enzyme on the spread of ink in estrogen-treated and gonadotropin = 0.002.

"p" value of difference between the effect of the enzyme on the spread of ink in normal and estrogen-treated = 0.10 (not significant).

TABLE 6

THE EFFECT OF ESTROGEN AND CHORIONIC GONADOTROPIN ON THE TISSUE AND VASCULAR PERMEABILITY

Normal				*Estrogen-treated*				*Gonadotropin-treated*			
rabbit number	*tissue permeability; spread of intracutaneous irritant in:*		*vascular permeability; intensity of color*	*rabbit number*	*tissue permeability; spread of intracutaneous irritant in:*		*vascular permeability; intensity of color*	*rabbit number*	*tissue permeability; spread of intracutaneous irritant in:*		*vascular permeability; intensity of color*
	15 minutes	*1 hour*			*15 minutes*	*1 hour*			*15 minutes*	*1 hour*	
A10-29	182	242	+++	A10-9	171	277	++	A10-11	292	338	+++
A10-42	231	337	+++	A10-31	158	249	++	A10-32	184	275	++++
A10-69	297	404	+++	A10-66	194	226	++	A10-67	219	413	++++
A10-82	225	329	++++	A10-80	151	206	+++	A10-81	147	250	++
A10-71	187	253	++	A10-72	154	212	+++	A10-72	500	626	+++++
Average ...	225	313	+++		166	234	++±		269	381	++++±

gonadotropin-treated litter mates. Immediately thereafter, trypan blue was injected intravenously. The site of intracutaneous injection of exudate became much bluer than the rest of the skin. This was evidently due to

extravasation of dye into the area of increased vascular permeability penetrated by the leucotaxin.

It may be seen in TABLE 6 that in estrogen-treated rabbits the exudate spreads over a smaller area than in control litter mates, whereas, in the rabbits under the influence of gonadotropin, the irritant spreads over a greater area. This can be readily explained as resulting from the reduced connective-tissue permeability in the estrogen-treated rabbits, on the one hand, and from the increased permeability of this tissue in gonadotropin-treated rabbits, on the other.

The intensity of color of these areas of trypan-blue extravasation, which it would seem, mirrors the amount of dye exuding from the vessels into the tissues, tends to be less in the estrogen-treated rabbits and more in the gonadotropin-treated animals than in the corresponding controls. This interpretation seems plausible, for this difference could not be accounted for by variations in the rate of removal of the dye from the site of extravasation by the tissues, as estrogen would aid its accumulation and gonadotropin would increase its diffusion.

Thus, all these observations on tissue and vascular permeability are in harmony with the observed restricting effect of estrogen on the dissemination of tuberculosis from the portal of entry in the skin and its enhancement by gonadotropin.

Summary

The localization of the disease at the portal of entry, which is characteristic of the response of genetically resistant rabbits to tuberculosis, and its dissemination from the portal of entry, which characterizes the disease in natively susceptible rabbits, can be simulated in rabbits of the same genetic resistance to the infection by exposing them to estrogen and chorionic gonadotropin, respectively. Estrogen retards the progress of tuberculosis at the portal of entry in the skin and diminishes its dissemination to the internal organs in highly inbred animals, chiefly by reducing the permeability of the connective tissue. Chorionic gonadotropin enhances the disease at the portal of entry, and its spread through the body, by increasing the permeability of the connective tissue.

Estrogen increases the turgidity of the connective tissue elements; gonadotropin tends to reduce it. Estrogen tends to reduce vascular permeability of the skin; gonadotropin appears to increase it. Estrogen has no effect on the multiplication and destruction of tubercle bacilli in the tissues, nor does either of the two hormones exercise any effect on antibody formation or on the intrinsic mechanism responsible for the development of allergic sensitivity, though estrogen reduces the inflammatory irritability of the skin to bacterial toxic agents in general. While estrogen depresses the circulating lymphocytes of the blood, the intermediation of the adrenal cortex in this effect has not been demonstrated. Estrogen also markedly spares the tissues from amyloid degeneration, but this property of the hormone is not significant in the retardation of the disease in the early phase of the infection by estrogen.

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